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Influence of Testicular Steroids on Thyrotropin Releasing Hormone-induced Prolactin Release in Mature Rams

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Prolactin (PRL) concentrations were measured in serum of intact rams, castrate rams, and steroid-treated castrate rams to determine whether testicular steroids influence PRL secretion in this species. Testosterone was administered to castrate rams by subdermal Silastic implants providing serum testosterone concentrations similar to those found in intact rams; estradiol was administered similarly to castrate rams, providing a ten-fold elevation of serum estradiol. Testosterone and estradiol implants decreased serum LH to concentrations found in intact rams, whereas estradiol, but not testosterone, effectively increased basal concentrations of PRL. This effect was most apparent when animals were exposed to short photoperiods (8 hours light:16 hours darkness). When 5 μ g of thyrotropin releasing hormone were injected into these animals, peak PRL concentrations were lowest in castrate rams and highest in estradiol-treated castrate rams. PRL responses in testosterone-treated castrate rams and intact rams to the releasing hormone were intermediate. Results for peak PRL concentrations and area under the PRL response curves were similar. In conclusion, testicular steroids are suggested to play an important role in the regulation of PRL secretion in mature rams.

Key words: testicular steroids, prolactin, prolactin secretion.

The physiologic requirement for prolactin (PRL) in male reproduction remains controversial, yet the presence of specific receptors (Aragona and Friesen, 1975; Barkey et al, 1979) in the testis and accessory sex glands implicates an important need for PRL. PRL has been shown to enhance the testosterone secretory response to hCG in normal men (Ambrosi et al, 1976) and potentiate the action of hCG on the growth of the ventral prostate in rats (Dattatreymurthy et al, 1975). Synergistic effects of PRL have been documented for other aspects of male reproduction (Bartke, 1971; Bex and Bartke, 1977). In spite of this information, little is known about the regulation of PRL secretion in males. Environmental factors such as day length (photoperiod) can induce sustained changes in blood PRL concentrations in rams (Pelletier, 1973; Schanbacher and Ford, 1979) and bulls (Bourne and Tucker, 1975; Leining et al, 1979); however, the influence of testicular secretions and steroid treatment have yet to be properly evaluated.

Pelletier (1973) studied the effects of castration and photoperiod exposure on plasma PRL concentrations in mature rams and reported significant differences due to photoperiod but not to castration or testosterone propionate treatment. Contrary to this finding, testosterone propionate reportedly stimulates PRL secretion in young castrate rams (Davis et al, 1978), rats (Herbert, 1978), and male rhesus monkeys (Herbert et al, 1977). Nolin et al (1977) suggested that the stimulatory effects of testosterone on PRL secretion are mediated through conversion to estrogen since

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administration of the nonaromatizable androgen, dihydrotestosterone, fails to stimulate PRL secretion. Findings in humans further support this conclusion because estrogen treatment elevates PRL secretion in normal men (Wiedemann et al, 1976) as well as hypogonadal (Yen et al, 1974) and agonadal (Judd et al, 1979) women. In view of these findings and the poorly understood variables affecting PRL release in sheep (Lamming et al, 1974), an evaluation of PRL secretion in intact, castrate, and steroid-treated castrate rams was conducted.

Materials and Methods

Animal Treatments

Sixteen intact rams were assigned to this study at approximately eight months of age. Four rams were left intact (group R) and 12 rams were castrated. Immediately after castration, four of these animals (group T) were implanted subdermally with five Silastic capsules (3.35 mm × 4.65 mm × 30 cm; Dow Corning Corp., Midland, Michigan) containing testosterone, and four (group E) were implanted similarly but with a single Silastic capsule (3.35 mm × 4.65 mm × 27 cm) containing estradiol-17 β . The remaining four were sham implanted to serve as castrate controls (group C). Following this treatment, the 16 animals were housed as a group in a controlled environment. Temperature was maintained near 18 C throughout the study, during which time the animals were exposed to a long (16 hours light:8 hours darkness) photoperiod for 12 weeks, followed by a short (8 hours light:16 hours darkness) photoperiod for an additional 12 weeks.

Blood Sampling

Single blood samples were collected from each animal during the 11th and 23rd week of study by jugular venipuncture. In addition to these samples, eight sequential samples were collected from indwelling jugular cannulae at 15-minute intervals during the last week of exposure to long and short photoperiods. To further test whether PRL secretion differed among these four treatment groups, PRL release in response to an intravenous dose of 5 μ g of thyrotropin releasing hormone (TRH) was determined for each animal during the last week of exposure to short photoperiod. Again, blood samples were collected from indwelling jugular cannulae at 15-minute intervals starting 30 minutes before the test injection and continuing for an additional 150 minutes.

Radioimmunoassay Procedures

Serum concentrations of LH, testosterone, and estradiol were determined by validated assay techniques (Schanbacher and Ford, 1976). Sensitivity of the respective assays was 0.5 ng/ml, 0.1 ng/ml, and 1 pg/ml. NIH-LH-S18 was used as the reference standard for serum LH. The procedures for determining serum PRL con-

centrations have been described elsewhere (Schanbacher and Ford, 1979). Each serum sample was assayed in duplicate within a single assay at either 12.5, 50, or 200 μ l. Thus, PRL concentrations could be determined between 1 ng/ml and 3200 ng/ml. The intra-assay coefficient of variation for all duplicates was generally less than 10% and never exceeded 20%. NIH-P-S11 was used as the reference standard for serum PRL.

Statistical Analysis

Hormone data were statistically examined by analysis of variance followed by Duncan's multiple range test (Steel and Torrie, 1960). Logarithmic transformation of the data was required to analyze the PRL responses to TRH injections because treatment means and variances were correlated; i.e., before transformation, the variances were heterogeneous.

Results

Serum hormone concentrations determined from samples collected by venipuncture are given in Table 1 for intact rams (R), castrate rams (C), testosterone-treated castrate rams (T), and estradiol-treated castrate rams (E). LH, PRL, and testosterone concentrations in rams were significantly affected by photoperiod. While PRL decreased ($P < 0.01$) during exposure to short photoperiod, LH and testosterone increased ($P < 0.05$). Castration had no apparent effect on PRL concentrations, but decreased ($P < 0.01$) testosterone and estradiol concentrations and increased ($P < 0.01$) LH concentrations. Testosterone and estradiol concentrations in implanted animals remained essentially unchanged between the 11th and 23rd week of the experiment, and PRL was not consistently affected by either treatment. Implants which maintained serum testosterone at approximately 4.3 ng/ml reduced LH concentrations to those observed in intact rams. A similar effect on LH was observed with estradiol implants; however, estradiol concentrations were elevated ($P < 0.01$) relative to intact rams to produce this effect (Table 1).

Concentrations of serum PRL determined from samples collected from indwelling jugular cannulae are given in Table 2. Although serum PRL differed between long and short photoperiods, castration and testosterone treatment had no significant effect on PRL secretion. Average serum PRL concentrations were elevated by estradiol treatment but only significantly so during exposure to short photoperiod.

The response of serum PRL to intravenous TRH injection is shown in Fig. 1 and Table 3. Preinjec-

TABLE 1. Serum Hormone Concentrations in Intact, Castrate, and Steroid-treated Castrate Rams during Exposure to Long (16:8) and Short (8:16) Photoperiods

Group	Photoperiod	LH (ng/ml)	PRL (ng/ml)	Testosterone (ng/ml)	Estradiol (pg/ml)
R	16:8	1.3 ± 0.1†	200 ± 23	2.5 ± 1.1†	2.4 ± 0.2†
	8:16	2.2 ± 0.2†	16 ± 6	6.3 ± 2.2†	2.3 ± 0.3*
C	16:8	14.3 ± 1.7	212 ± 18	0.3 ± 0.1	1.2 ± 0.1
	8:16	19.1 ± 3.3	33 ± 10	0.2 ± 0.1	1.2 ± 0.2
T	16:8	1.0 ± 0.1†	193 ± 18	4.4 ± 0.4†	2.7 ± 0.3†
	8:16	1.5 ± 0.3†	15 ± 7	4.2 ± 0.6†	1.6 ± 0.3*
E	16:8	2.3 ± 0.3†	267 ± 25	0.3 ± 0.1	21.4 ± 2.2†
	8:16	2.5 ± 0.2†	32 ± 5	0.3 ± 0.1	22.3 ± 1.6†

Values are $\bar{X} \pm \text{SEM}$ for four animals. Blood samples were collected by jugular venipuncture.

* $P < 0.05$, † $p < 0.01$. Significantly different from castrate value within the same photoperiod.

R = intact; C = castrated, sham implanted controls; T = castrated, subdermal implantation of five Silastic capsules containing testosterone; E = castrated, subdermal implantation of a single Silastic capsule containing estradiol-17 β .

tion PRL concentrations were elevated ($P < 0.05$) in estradiol-treated animals. PRL concentrations increased in each animal given 5 μg of TRH; however, the responses were least in nonimplanted castrate rams. The peak responses in intact rams were variable, whereas the peak responses of estradiol-treated animals were more uniform and of greater magnitude. The strong influence of estradiol on TRH-induced PRL release is further illustrated by the area under the response curve (Table 3).

Discussion

From the present investigation it can be concluded that testicular steroids influence PRL secretion; however, this effect is less pronounced

than that due to photoperiod. Seasonal variation in blood PRL of rams (Ravault, 1976) has been shown to be regulated by day length (Pelletier, 1973; Lincoln et al, 1978; Schanbacher and Ford, 1979). Based on the data presented herein, estradiol enhances PRL secretion in castrate rams, but the results are convincing only during exposure to short day lengths. Perhaps the elevated concentrations of PRL during exposure to long day lengths partially mask the stimulatory effects of estradiol. Another possible explanation for the variable PRL concentrations reported in this study is that blood samples collected by jugular venipuncture are not useful to accurately reflect PRL secretory patterns. Because stress-induced changes in serum PRL have been described previously (Raud et al, 1971), only those PRL values

TABLE 2. Prolactin Secretion in Intact, Castrate, and Steroid-treated Castrate Rams during Exposure to Long (16:8) and Short (8:16) Photoperiods

Group	Photoperiod	Serum PRL Concentration (ng/ml)		
		Average	Minimum	Maximum
R	16:8	175 ± 18	135 ± 11	216 ± 25
	8:16	36 ± 10	21 ± 7	50 ± 16
C	16:8	172 ± 15	138 ± 10	204 ± 23
	8:16	29 ± 8	18 ± 3	41 ± 14
T	16:8	164 ± 17	130 ± 11	197 ± 25
	8:16	52 ± 24	25 ± 9	78 ± 39
E	16:8	216 ± 45	135 ± 9	297 ± 82
	8:16	129 ± 37*	64 ± 7*	194 ± 89

Values are $\bar{X} \pm \text{SEM}$ for four animals. Blood samples from each animal were collected from indwelling jugular cannulae.

* $P < 0.05$. Significantly different from castrate value within the same photoperiod.

R = intact; C = castrated sham implanted controls; T = castrated, subdermal implantation of five Silastic capsules containing testosterone; E = castrated, subdermal implantation of a single Silastic capsule containing estradiol-17 β .

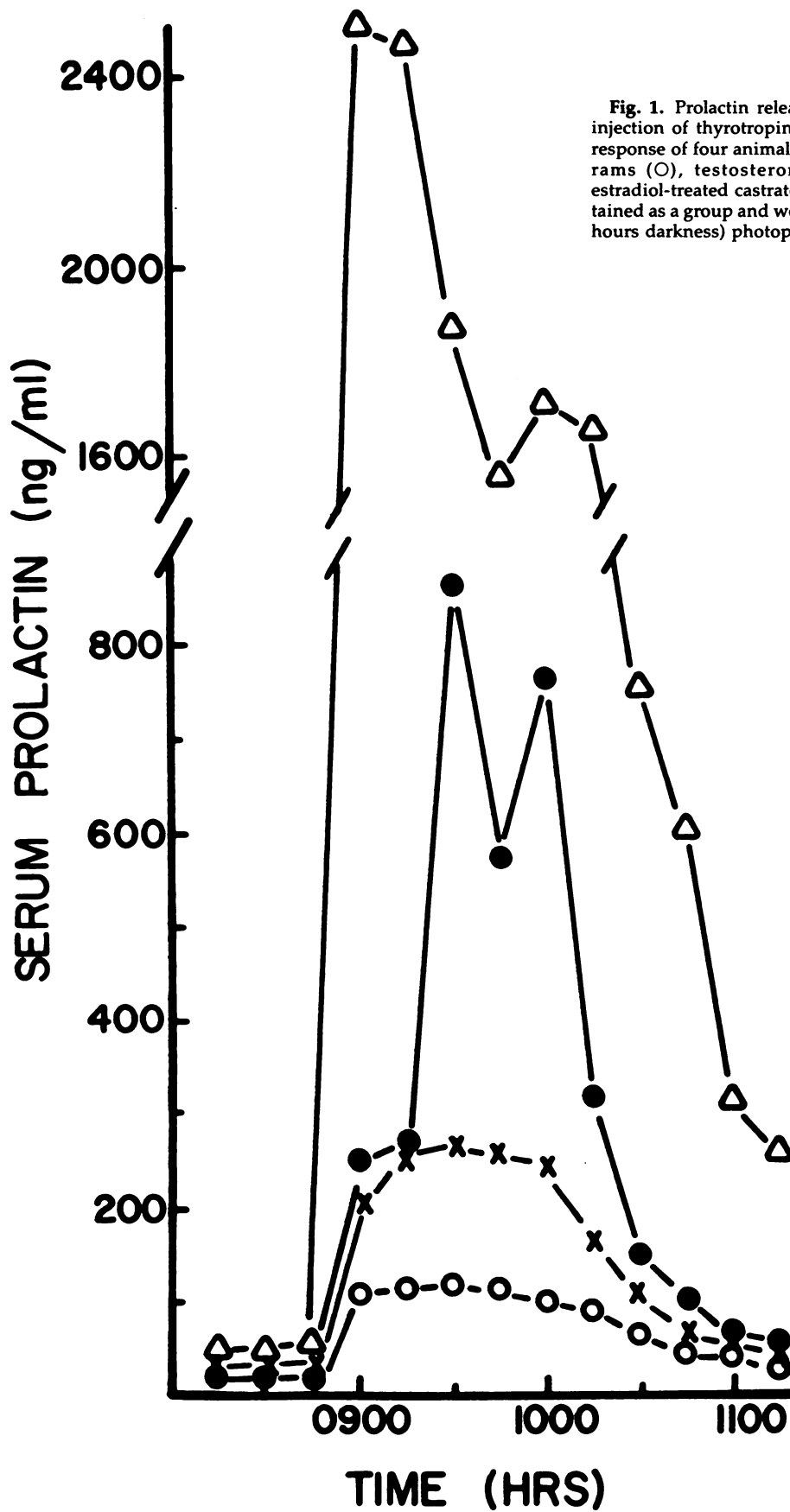


Fig. 1. Prolactin release in response to a 5- μ g intravenous injection of thyrotropin releasing hormone (TRH). The mean response of four animals is shown for intact rams (●), castrate rams (○), testosterone-treated castrate rams (x), and estradiol-treated castrate rams (Δ). These animals were maintained as a group and were exposed to a short (8 hours light : 16 hours darkness) photoperiod at the time of TRH challenge.

TABLE 3. Response of Serum Prolactin to a 5- μ g Intravenous Injection of Thyrotropin Releasing Hormone in Intact, Castrate, and Steroid-treated Castrate Rams during Exposure to Short (8:16) Photoperiods

Group	Preinjection Value	Peak Value	Area under Response Curve*
R	22 \pm 8	907 \pm 765	43 \pm 32
C	17 \pm 7	130 \pm 42	9 \pm 3
T	34 \pm 9	299 \pm 161	21 \pm 11
E	54 \pm 12†	2847 \pm 352‡	172 \pm 32‡

Values are $\bar{X} \pm$ SEM for four animals. Blood samples from each animal were collected from indwelling jugular cannulae.

* Area determined by planimetry of the plotted data (arbitrary units).

† $P < 0.05$, ‡ $P < 0.01$. Significantly different from castrate value.

R = intact; C = castrated, sham implanted controls; T = castrated, subdermal implantation of five Silastic capsules containing testosterone; E = castrated, subdermal implantation of a single Silastic capsule containing estradiol-17 β .

determined for samples collected from indwelling jugular cannulae (Tables 2 and 3) should be emphasized.

The pattern of PRL secretion is shown to be irregular and pulsatile in male rats even when baseline PRL concentrations are elevated by estradiol (Shin and Chi, 1979). The larger difference between minimum and maximum PRL concentrations of estradiol-treated castrate rams suggests an influence of estrogen on synthesis, storage, and magnitude of episodic PRL release. The response of estradiol-treated animals to a TRH challenge was determined to test this possibility. As shown by Debeljuk et al (1973) and Malven (1979), TRH effectively releases PRL into the circulation. The enhanced response of estradiol-treated animals to TRH shows a positive influence of estradiol on PRL. Increased plasma PRL concentrations at the time of estrus in sheep (Lamming et al, 1974) and the positive influence of estrogen infusion on plasma PRL concentration in cattle (Schams and Reinhardt, 1973) support the conclusion that estrogens in domestic animals are important in the regulation of PRL secretion (Padmanabhan and Convey, 1979).

The importance of testicular steroids and, in particular, estrogen on PRL synthesis and release is best established for rodents (Herbert et al, 1977; Maurer and Gorski, 1977; Vaughan et al, 1978; Shin and Chi, 1979; Sinha et al, 1979). Nolin et al (1977) suggested that the positive effects of testosterone on PRL release could be due to aromat-

ization products. The higher serum concentrations of estradiol in intact rams and testosterone-treated castrate rams as compared to untreated castrate rams may explain the higher PRL response of these animals to TRH. On the other hand, similarity of serum estradiol concentrations and variability in PRL responses of these animals to TRH is difficult to interpret. Castrate male mice have reduced serum and pituitary concentrations of PRL and a decreased PRL response to perphenazine relative to intact mice (Sinha et al, 1979). PRL secretion in these mice is enhanced more effectively by estradiol benzoate treatment than by testosterone treatment. Treatment of castrate rams with testosterone propionate or diethylstilbestrol (DES) was shown to increase mean plasma PRL concentrations and amplitude of PRL secretory spikes (Davis et al, 1978). Interestingly, these investigators observed a stimulatory effect of DES on basal PRL concentrations, whereas testosterone propionate was ineffective. Stimulation of basal PRL was observed with estradiol (but not testosterone) in this study. Pelletier (1973) reported no stimulatory effect of testosterone propionate treatment on PRL secretion in mature castrate and intact rams. These different results indicate that the method of steroid administration and extent of PRL secretory analysis are important relative to the interpretation of steroid influences on PRL secretion in sheep.

In conclusion, several factors influence PRL secretion in rams. Those studied herein include day length, hypothalamic releasing hormone, and gonadal steroids. Gonadal regulation of LH and PRL secretion and their inverse relationship in sheep may provide important insight relative to the mechanisms which control seasonal breeding in this species.

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